Journal of Medical Virology 60:347–352 (2000)

Immunoreactivity Against Linear Epitopes of Parvovirus B19 Structural Proteins. Immunodominance of the Amino-Terminal Half of the Unique Region of VP1

Monica Musiani,* Elisabetta Manaresi, Giorgio Gallinella, Simona Venturoli, Elisa Zuffi, and Marialuisa Zerbini

Department of Clinical and Experimental Medicine, Division of Microbiology, University of Bologna, Italy

Three peptides corresponding to the 2-100 amino acids of VP1 unique sequence (VP1-F1), to the 99-227 amino acids of VP1 unique sequence (VP1-F2) and to the 237-781 amino acids of VP1 protein common to VP2 (VP1-F3 = VP2) were produced by prokaryotic expression. The three peptides, which span the entire VP1 structural protein of parvovirus B19 and also the entire VP2 protein, were used to evaluate the immunoreactivity against linear epitopes of these fragments in a large number of serum samples taken in different clinical situations with regards to B19 infection and in some commercial preparations of aspecific immunoglobulins. The data demonstrated that the specific VP1-F1 fragment, corresponding to the amino-terminal half of the VP1 unique region, is immunodominant and can elicit a long lasting immune response in comparison with VP1-F2 and VP1-F3 = VP2. Data regarding the presence of specific IgG to the three fragments in commercial preparations of immunoglobulins demonstrated that the dominant immune response was also against VP1-F1 linear epitopes while IgG against VP1-F2 and IgG against VP1-F3 = VP2 could be found only in high concentrations of Ig preparations. The reported data can be useful as a basis for the development of a B19 recombinant vaccine. ***J. Med. Virol. 60:347–352, 2000.*** © 2000 Wiley-Liss, Inc.

KEY WORDS: recombinant peptides; VP1 unique portion; immunoglobulin preparations

INTRODUCTION

Human parvovirus B19 is associated with a wide range of acute clinical manifestations such as erythema infectiosum, post-infectious arthropathy, transient aplastic crisis in patients with chronic hemolytic anemias and fetal hydrops. Moreover, parvovirus B19 may cause persistent infections, mainly in immunocompromised patients generally leading to chronic bone marrow failure resulting in severe chronic anemia [Zerbini and Musiani, 1999].

In immunocompetent individuals, the development of a specific immune response correlates with clearance of viremia [Musiani et al., 1995] and generally with the prevention of reinfection, although in some cases reinfections have been described in the presence of a low level of specific IgG.

In immunocompromised patients, B19 specific immune response may be normal, altered and even absent and in these patients immunoglobulin therapy has shown to clear the virus from the circulation or to control the clinical course of B19 persistent infections. The immune response against B19 is directed mainly to the viral capsid proteins. Capsids are composed of two proteins, VP1 of 83 kDa and VP2 of 58 kDa, which represent about 4 and 96% of the capsid respectively [Ozawa and Young, 1987]. VP1 and VP2 derive from overlapping reading frames and are identical except that VP1 contains an unique region of 227 additional amino acids at the amino-terminus [Ozawa et al., 1987].

The study of the immune response against B19 proteins has been hampered for several years by the limited availability of native antigens, since isolation of viral particles as a source of antigens relies on the detection of viremic blood units by screening blood donors. To overcome the shortage of B19 antigens, recombinant B19 proteins have been expressed in several systems, both procaryotic and eukaryotic [Morinet et al., 1989; Brown et al., 1990; Kajigaya et al., 1991; Söderlund et al., 1992; Manaresi et al., 1999].

Grant sponsor: CNR target project on Biotechnology; Grant sponsor: University of Bologna Funds for selected research topics; Grant sponsor: MURST.

*Correspondence to: Prof. Monica Musiani, M.D, Division of Microbiology, Via Massarenti 9, 40138 Bologna, Italy. E-mail: musiani@med.unibo.it

Accepted 21 July 1999

TABLE I. Primers Used for Amplification of Regions Coding for Recombinant Proteins

Primer	Position	Sequence (5′-3′)
c2630	2450–2469	GGGAAGCTTCA-AAAGAAAGTGGCAAATGGTG
c2921	2741–2758	GGGAAGCTTCA-CATGCAGAACCTAGAGGA
c2923	2743–2723	GGGGCGGCCGCTTAGTGATGGTGATGGTGATGTCTAGA-ATGACTGCTACTGGATGATAA
c3310	3124–3107	GGGGCGGCCGCTTAGTGATGGTGATGGTGATGTCTAGA-GCTTGGGTATTTTTCTGA
c3335	3155–3172	GGGAAGCTTCA-ACTGGTGCAGGAGGGGGT
c4966	4786–4769	GGGGCGGCCGCTTAGTGATGGTGATGGTGATG-CAATGGGTGCACACGGCT

Recently, several studies have demonstrated that parvovirus B19 IgG have a different reactivity against conformational and linear epitopes of VP1 and VP2 [Söderlund et al., 1995; Kerr et al., 1999; Manaresi et al., 1999]. As regards immunoreactivity against conformational epitopes of B19 VP1 and VP2, the presence of specific IgG is essentially uniform for both the structural proteins during all the phases of B19 infection [Söderlund et al., 1995; Kerr et al., 1999]. As regards the immunoreactivity against linear epitopes, specific IgG to VP1 and VP2 are concomitantly present during the active/very recent and early convalescent phases of B19 infection while in past infection the immune response is directed mainly against VP1 linear epitopes [Söderlund et al., 1995; Manaresi et al., 1999].

Neutralizing regions have been demonstrated both in the VP1 and VP2 proteins and most of the neutralizing epitopes in the VP1 unique region appear to be linear in contrast to those in VP2 which appear to be conformational [Saikawa et al., 1993; Kajigaya and Momoeda, 1997]. A previous study using synthetic peptides from the VP1 unique region demonstrated that the regions which elicit a neutralizing activity when injected into rabbits are located within the first 80 amino acids and within amino acids 148-205 of VP1 [Anderson et al., 1995]. All these data prompted the analysis of the specific IgG immune response in humans to different linear epitopes of VP1 structural protein using recombinant fragments corresponding to the regions that were demonstrated to elicit a neutralizing activity in rabbits. Three recombinant peptides, spanning the entire VP1 and also VP2, corresponding to the 2-100 amino acids of VP1 unique sequence (VP1-F1), the 99-227 amino acids of VP1 unique sequence (VP1-F2) and to the 237-781 amino acids of VP1 protein common to VP2 (VP1-F3 = VP2) were produced by a procaryotic expression system. The three fragments were used to evaluate the IgG humoral response against linear epitopes by Western blot assay in a large number of immunocompetent subjects with different clinical situations and different laboratory evaluations with regards to B19 infection. Since aspecific immunoglobulin preparations are used commonly to clear parvovirus B19 from infected individuals, the presence of specific IgG to linear epitopes of B19 structural proteins was also sought in different commercial immunoglobulin preparations.

MATERIALS AND METHODS

Recombinant Fragments Production

The vector used for *Escherichia coli* expression of the recombinant fragments, was constructed by transferring the Hpa I-Sca I sequence from PinPoint Xa-1 Vector (Promega Corporation,WI), containing the tac promoter and a sequence for a fusion biotinylated 13KDa peptide, into Eco 47 III- and Sca I- digested pBR322. Three B19 DNA regions (343 bp, 433 bp and 1672 bp) coding respectively for: (a) the 2-100 amino acids of VP1 unique region; (b) the 99-227 amino acids of VP1 unique region; and (c) the 237-781 amino acids of VP1 protein common to VP2, were obtained by PCR amplification of a plasmid containing the complete internal unique sequence of parvovirus B19 DNA (pB19-A) [Gallinella et al., 1993]. Amplification primer sequences were designed to obtain Hind III restriction sites as a part of the upstream primers and Not I restriction sites as a part of the downstream primers (Table I). The downstream primers were synthesized with a sequence coding for six histidines at the 3′-end to facilitate subsequent purification by metal chelate affinity chromatography. Primers c2630 and c2923 were used for the amplification of the DNA region coding for the 2-100 amino acids of VP1 unique region (VP1-F1), primers c2921 and c3310 were used for the amplification of the DNA coding region for the 99-227 amino acids of VP1 unique region (VP1-F2) and primers c3335 and c4966 were used for the amplification of the DNA region coding for the 237- 781 amino acids of VP1 protein common to VP2 (VP1-F3 = VP2).

Polymerase chain reaction (PCR) was carried out by the hot start method using Ampliwax gems (Perkin Elmer Italia SPA, Monza, Italy) in 100 μl of TrisCl 10 mM pH 8.8, KCl 25 mM, $(NH_4)_2SO_4$ 5 mM, $MgSO_4$ 3 mM. For each reaction 100 ng of parvovirus B19 insert purified from pB19-A DNA, 200 μM of each dNTP, 0.1 μM of each primer and 2.5 Units of Pwo DNA Polymerase (Boehringer, Mannheim, Germany) were used. The DNA was amplified in 40 cycles, each consisting of denaturation for 30 sec at 95°C, annealing for 30 sec at 52°C and extension for 2 min at 72°C. Prior to amplification the reaction mixture was kept for 5 min at 95°C; an additional 5 min was added to the final 72°C elongation cycle.

All amplification products were purified by Qiaquick PCR Purification kit (Qiagen Inc., CA) and then cleaved with Hind III and Not I. The cleaved fragments

were purified by Qiaquick Nucleotide Removal Kit (Qiagen Inc., Valencia, CA) and then ligated to Hind III-Not I digested vector; the ligation mixtures were used to transform *E. coli* DH5α competent cells by standard procedures. Selected colonies were grown and screened to identify clones containing recombinant plasmids with VP1 fragments coding regions. Clones pHV1-F1, pHV1-F2, and pHV1-F3, respectively, were thus obtained.

Protein Expression

Overnight cultures of cells harboring the recombinant plasmids were diluted 1:20 in LB-ampicillin and grown for 1 h in a shaking incubator at 37°C. Expression of the recombinant fusion proteins was induced by addition of 100 μM IPTG for 5 h at 37°C. The induced cells were centrifuged and stored in aliquots at –20°C. Aliquots of each recombinant bacteria were resuspended in sample buffer (glycerol 10%, mercaptoethanol 5%, SDS 2%, 1 mg/ml bromophenol blue in TrisCl 60 mM pH 6.8), heated at 95°C for 5 min and tested for the presence of recombinant fragments by SDS-PAGE (8% acrylamide) followed by staining with Comassie Brillant Blue.

Purification of Recombinant Proteins

The cell pellets were lysed in Lysis buffer (TrisCl 20 mM pH 8, NaCl 100 mM, GuHCl 6 M, Triton 0.1%, Imidazole 3 mM). After 30 min incubation and 3 cycles of freeze-thawing, the cellular lisates were loaded on TALON metal affinity spin columns (Clontech Laboratories, Inc., Palo Alto, CA). The columns were washed twice with Wash buffer (TrisCl 20 mM pH 8, NaCl 100 mM, Urea 8 M, Imidazole 15 mM) and eluted with Elution Buffer (TrisCl 20 mM pH 8, NaCl 100 mM, Urea 8 M, Imidazole 100 mM). Fractions were tested for the recombinant protein by SDS-PAGE (8% acrylamide) followed by staining with Comassie Brillant Blue.

Samples

Human sera to be tested by Western blot to detect IgG antibodies to linear epitopes of recombinant fragments VP1-F1, VP1-F2, and VP1-F3 = VP2 were chosen from different patients with different clinical situations and laboratory evaluations with regards to B19 infection. A total of 109 serum samples from 105 patients were examined. All the patients involved in the study were aged between 10 and 63 years and apparently had no underlying immunological diseases. Immunocompromised patients, such as human immunodeficiency virus (HIV) infected subjects, patients with leukemia and transplant recipients, were excluded from the study. All the sera had been examined previously for the presence of B19 DNA using dot blot hybridization and nested PCR, carried out as described previously [Zerbini et al.,1990; Gallinella et al., 1993] and for the presence of specific IgM and IgG against VP2 conformational epitopes using commercial ELISA commercial assays (IDEIA, Parvovirus B19, IgM and IDEIA, Parvovirus B19, IgG by Dako A/S, Glostrup, Denmark).

The samples were as follows. Seven serum samples from 7 patients with a B19 acute infection at the onset of symptoms, positive for B19 DNA with or without specific IgM but without IgG against VP2 conformational epitopes at ELISA. This group consisted of 5 patients with aplastic crisis, 1 patient with thrombocytopenia and neutropenia, and 1 patient with fever. Twenty-three serum samples from 23 patients with a symptomatic B19 active or very recent infection diagnosed by the presence of B19 DNA and/or IgM and of IgG against VP2 conformational epitopes at ELISA. This group consisted of ten patients with rash, seven with bone marrow hypoplasia and six with arthritis. Nine serum samples from 5 convalescent phase patients, negative for B19 DNA and IgM but positive for IgG against VP2 conformational epitopes by ELISA. Sera were collected between 3 and 8 months after a documented acute symptomatic B19 infection. Fifty-seven serum samples sent to the laboratory without a specific request of B19, showing a past B19 infection i.e. absence of B19 DNA and IgM and presence of IgG against VP2 conformational antigens at ELISA. Thirteen sera collected during a non-epidemic period (January–March 1997) from 13 controlled blood donors without IgG immunity against VP2 conformational antigens and also negative for B19 DNA and specific IgM. The blood donors did not have B19 DNA, specific IgM and IgG against VP2 conformational epitopes at ELISA during the last three years.

Four different commercial immunoglobulin preparations were tested by Western blot for the presence of specific IgG to linear epitopes of recombinant fragments VP1-F1, VP1-F2, and VP1-F3 = VP2. The Ig commercial preparations were Sandoglobulina (Sandoz Pharma S.A., Basel, Switzerland), Ig Vena N.I.V. (Sclavo, Siena, Italy), Endobulin (Immuno S.p.A., Pisa, Italy), Globuman Berna (Swiss Serum and Vaccine Institute, Berna, Switzerland). Different Ig concentrations (500 μg/ml, 250 μg/ml, 125 μg/ml, 62.5μg/ml, 31.2 μg/ml, 15.6 μg/ml, 7.8 μg/ml, 3.9 μg/ml, 1.9 μg/ml) were tested for each preparation.

Western Blot

The purified fragments were loaded in equal amounts and were separated under denaturing conditions by SDS-PAGE (8% acrylamide). The fragments were then transferred to a nitrocellulose membrane by using a Mini Trans-Blot Electrophoretic Transfer cell (Bio-Rad Laboratories, Milan, Italy) and the membrane was cut longitudinally into strips which were 2 mm wide. Each strip was treated with Blocking buffer (1% dried milk in NaCl 150 mM, TrisCl 100 mM pH 7.5) for 2 h and then incubated for 1 h with 1.5 ml of human sera (1:100 in Blocking buffer) or with 1.5 ml of the different concentrations of commercial Ig preparations in Blocking buffer. After washing with PBS-Tween 20 0.3%, the strips were incubated for 1 h with

TABLE II. B19 Virological and Serological Testings in Serum Samples Taken From Subjects in Different Phases of B19 Infection

Phase of B19 infection	Number of sera examined	Number of serum samples positive at: B19DNA/IgM	IgG anti conformational epitopes	IgG anti VP1-F1 linear epitopes	IgG anti VP1-F2 linear epitopes	IgG anti VP1-F3 = VP2 linear epitopes
Acute at the onset of symptoms	7	7	0	0	0	0
Active or very recent	23	23	23	23 (100%)	12 (52.2%)	21 (91.3%)
Convalescent (3–8 months after infection)	9	0	9	9 (100%)	9 (100%)	9 (100%)
Past infection	57	0	57	44 (77.2%)	9 (15.8%)	12 (21.1%)
Absence of a documented prior infection	13	0	0	1	1	1

peroxidase-conjugated anti human IgG (Dako A/S, Glostrup, Denmark) diluted 1:1000 in Blocking buffer. The membranes were then incubated in the dark with the enzyme substrate for peroxidase consisting of a solution of 2 ml methanol containing 6 mg of 4-chloro-1-naphtol (Bio-Rad Laboratories, Milan, Italy) added immediately before use to a solution of 10 ml of PBS containing 6 μl of 10% hydrogen peroxide. Membranes were washed in distilled water, air dried and stored for permanent record. The optimal working dilution of each immune reagent used in the reaction was determined by preliminary block titration.

RESULTS

Three fragments corresponding to the 2-100 amino acids of VP1 unique region (VP1-F1), the 99-227 amino acids of VP1 unique region (VP1-F2) and the 237- 781 amino acids of VP1 protein common to VP2 (VP1-F3 = VP2) respectively were cloned and expressed in *E. coli*. Recombinant fragments expressed as fusion proteins were then purified by metal chelate affinity chromatography; SDS-PAGE analysis of purified recombinant proteins revealed bands of 25.8, 28.3, and 71 KDa respectively, corresponding to the expected size of the three fragments fused with the 13 KDa peptide. Purified proteins were then used in Western blot assays to detect specific IgG antibodies present in serum samples and in commercial Ig preparations.

The results obtained from the analysis of serum samples obtained from patients in different phases of B19 infection are summarized in Table II. In the group of patients with B19 acute infection at the onset of symptoms, out of 7 serum samples from 7 patients who had B19 DNA with or without specific IgM but without IgG against VP2 conformational epitopes, none was positive for IgG against linear epitopes of VP1-F1, VP1-F2 and VP1-F3 = VP2.

In the group of 23 patients with an active or very recent symptomatic B19 infection, out of 23 samples (positive for B19 DNA and/or IgM and positive for IgG against VP2 conformational epitopes), all (100%) had IgG against linear epitopes of VP1-F1, 12 (52.2%) were positive for IgG against linear epitopes of VP1-F2 and 21 (91.3%) proved positive for IgG against linear epitopes of VP1-F3 = VP2. All 9 sera from convalescent–phase patients (negative for B19 DNA and IgM but positive for IgG against VP2 conformational epitopes) had IgG against linear epitopes of VP1-F1, VP1-F2, VP1-F3 = VP2.

In the group of 57 subjects with signs of a past B19 infection (negative for B19 DNA and IgM but positive for IgG against VP2 conformational epitopes) 44 (77.2%) had IgG against linear epitopes of VP1-F1, 9 (15.8%) were positive for IgG against linear epitopes of VP1-F2 and 12 (21.1%) had IgG against linear epitopes of VP1-F3 = VP2. Past infection sera which were negative for IgG against linear epitopes of VP1-F1, were also negative for IgG against linear epitopes of VP1-F2 and of VP1-F3 = VP2.

In the group without a preexisting immunity against VP2 conformational epitopes, out of 13 tested sera, 12 proved negative for IgG against linear epitopes of VP1-F1, VP1-F2 and VP1-F3 = VP2 while one proved positive for IgG against all the three fragments. To check if the discrepant sample was a real positive it was retested by Western blot with the entire VP1 [Manaresi et al.,1999] and found positive.

The presence of specific IgG against VP1-F1, VP1-F2 and VP1-F3 = VP2 was evaluated with four different commercial immunoglobulin preparations. Different concentrations (from 500 μg/ml to 1.9 μg/ml) for each preparation were tested and the lowest concentrations containing specific IgG against the three fragments are shown in Table III.

IgG against linear epitopes of VP1-F1 were found in the Ig concentration of 31.2 μg/ml in three preparations and in the Ig concentration of 15.6 μg/ml in the fourth. IgG anti VP1-F2 were found in only two preparations in the Ig concentration of 250 μg/ml while they were absent in the more concentrated specimens of 500 μg/ml in the other two. IgG anti VP1-F3 = VP2 were found in all the preparations in concentrations ranging between 250–125 μg/ml.

DISCUSSION

Fragments corresponding to the 2-100 amino acids of VP1 unique region (VP1-F1), to the 99-227 amino acids of VP1 unique region (VP1-F2) and to the 237-781 amino acids of VP1 protein common to VP2 (VP1-F3 = VP2) were produced. These fragments, which span the

TABLE III. Presence of Specific IgG Against VP1-F1, VP1-F2 and VP1-F3 = VP2 in Different Concentrations (From 500 μg/ml to 1.9 μg/ml) of Four Commercial Immunoglobulin Preparations

	Lowest concentration of Ig/ml positive at		
Commercial Ig preparation	IgG anti VP1-F1 linear epitopes	IgG anti VP1-F2 linear epitopes	IgG anti VP1-F3 = VP2 linear epitopes
Sandoglobulina	15.6 μg/ml	Neg	250 μg/ml
Ig Vena N.I.V.	31.2 μg/ml	250 μg/ml	250 μg/ml
Endobulin	31.2 μg/ml	Neg	125 μg/ml
Globuman Berna	31.2 μg/ml	250 μg/ml	125 μg/ml

entire VP1 protein were produced to evaluate if a portion of VP1 is immunodominant over the others as regards linear epitopes. The proteins were used as antigens in a Western blot assay to detect specific IgG in 109 serum samples from 105 different subjects without underlying immunologic diseases and with different clinical situations and laboratory evaluations with regards to B19 infection. Immunocompromised patients were excluded from the study since in these patients an altered or absent B19 specific immune response has been documented frequently.

IgG against linear epitopes of VP1-F1 appeared in all patients during the active or very recent phase of infection and in the convalescent phase (i.e. 3–8 months after acute infection). In past infection sera, determined by the presence of IgG against conformational antigens, IgG against linear epitopes of VP1-F1 were present in 77.2% of subjects and thus with a prevalence concordant with that observed previously for IgG to the linear epitopes of the entire VP1 [Manaresi et al., 1999.] Subjects without IgG against linear epitopes of VP1-F1, were also without IgG to VP1-F2 and VP1-F3 = VP2.

IgG against linear epitopes of VP1-F2 were detectable in half of the patients during the active or very recent phase of infection and in all the sera tested from patients in the convalescent phase suggesting a delay in the appearance of these antibodies in comparison with IgG against linear epitopes of VP1-F1. IgG against linear epitopes of VP1-F2 were present in a small percentage of individuals with a past infection (15.8%) suggesting a short lasting immune response against the second half of the VP1 unique portion.

IgG against linear epitopes of VP1-F3 = VP2 appeared in almost all patients during the active or very recent phase of infection and in all the sera tested from patients in the convalescent phase. In past infection sera, IgG against linear epitopes of VP1-F3 = VP2 were present in a small percentage of individuals (21.1%) confirming previous data on a low reactivity of past infection sera with VP2 linear epitopes [Söderlund et al., 1995; Kerr et al., 1999; Manaresi et al.,1999].

The importance of VP1 linear epitopes, in an effective immune response against B19, has been shown in several studies demonstrating that the dominant immune response of past infection human sera in immunoblots is directed to VP1 [Schwartz et al., 1988; Kurtzman et al., 1989; Söderlund et al.,1995; Palmer et al., 1996; Manaresi et al.,1999] and that patients with persistent parvovirus infections lack antibody able to react with linear epitopes of B19 structural proteins [Kurtzman et al., 1989]. The data demonstrate that among the entire VP1 protein, the specific VP1-F1 fragment, corresponding to the amino-terminal half of the VP1 unique region, is immunodominant and can elicit a long lasting immune response in humans in comparison with VP1-F2 and VP1-F3 = VP2. The presence of the amino-terminal half of VP1 unique region seems therefore very important in the production of recombinant antigens to use for immunoblot assays to detect a prior contact with B19.

Data on the presence of specific IgG to the three fragments in commercial preparations of immunoglobulins demonstrated that IgG against VP1-F1 linear epitopes were dominant while IgG against VP1-F2 and IgG against VP1-F3 = VP2 could be found only in high concentrations of Ig preparations. The results with our data in commercial immunoglobulins confirmed the data obtained with past infectious sera, since commercial immunoglobulins are obtained from pools of at least one thousand subjects and about 65% of adult population results with a B19 past immunity. Moreover, since commercial Ig preparations have been used successfully to clear the virus from circulation or to control the clinical course of B19 persistent infections [Anderson, 1997], it is speculated that the IgG immune response against the amino-terminal half of the VP1 unique region is important in virus neutralization and can therefore have a protective role.

Previous studies have demonstrated that multiple neutralizing epitopes are present both in VP1 and VP2 with those on VP1 unique region appearing to be linear in contrast to those on VP2 which appeared to be conformational [Saikawa et al.,1993; Kajigaya and Momoeda, 1997]. The data on a strong and long lasting antibody recognition of linear epitopes within the amino-terminal half of the VP1 unique region stress the role of the linear neutralizing epitopes found in this portion [Anderson et al., 1995] for an effective immune response in humans. Moreover the immunodominancy of the amino terminal half of the VP1 unique region together with the presence of neutralizing epitopes, can suggest that this portion is located externally to the capsid. This finding therefore agrees with works that have suggested that almost all of the VP1 unique re-

gion is located externally on the virions [Rosenfeld et al., 1992; Kawase et al., 1995].

The importance of VP1 for the development of B19 vaccine has been shown in several studies. In fact, in order to elicit antibody response in animals, recombinant empty capsids must contain VP1 since recombinant capsids consisting only of VP2 fail to elicit a neutralizing immune response in animals [Kajigaya et al., 1991] and the effective immune response is proportional to the quantity of VP1 contained in the empty capsid immunogen [Bansal et al., 1993]. These data therefore can also be useful as a basis for the development of a B19 recombinant vaccine either consisting of empty capsids enriched with the amino-terminal half of VP1 unique region or consisting of a mixture of several neutralizing and immunodominant peptides including the one corresponding to the first 100 amino acids of the VP1 unique region.

REFERENCES

Anderson LJ. 1997. Treatment and prevention of human parvovirus B19 disease. In: Anderson LJ, Young NS, editors. Human parvovirus B19. Monographs in Virology, Vol. 20. Basel: Karger. p 137–148.

Anderson S, Momoeda M, Kawase M, Kajigaya S, Young NS. 1995. Peptides derived from the unique region of B19 parvovirus minor capsid protein elicit neutralizing antibodies in rabbits. Virology 206:626–632.

Bansal GP, Hatfield JA, Dunn FE, Kramer AA, Brady F, Riggin CH, Collett MS, Yoshimoto K, Kajigaya S, Young NS. 1993. Candidate recombinant vaccine for human B19 parvovirus. J Infect Dis 167: 1034–1044.

Brown CS, Salimans MMM, Noteborn MHM, Weiland HT. 1990. Antigenic parvovirus B19 coat proteins VP1 and VP2 produced in large quantities in a baculovirus expression system. Virus Res 15:197–212.

Gallinella G, Musiani M, Zerbini M, Gentilomi G, Gibellini D, Venturoli S, La Placa M. 1993. Efficient parvovirus B19 DNA purification and molecular cloning. J Virol Methods 1:203–212.

Kajigaya S, Fujii H, Field A, Anderson S, Rosenfeld S, Anderson LJ, Shimada T, Young NS. 1991. Self assembled B19 parvovirus capsids, produced in a baculovirus system, are antigenically and immunogenically similar to native virions. Proc Natl Acad Sci USA 88:4646–4650.

Kajigaya S, Momoeda M. 1997. Immune response to B19 infection. In: Anderson LJ, Young NS, editors. Human parvovirus B19. Monographs in Virology, Vol 20. Basel: Karger. p 120–136.

Kawase M, Momoeda M, Young NS, Kajigaya S. 1995. Most of the VP1 unique region of B19 parvovirus is on the capsid surface. Virology 211:359–366

Kerr S, O'Keeffe G, Kilty C, Doyle S.1999. Undenatured parvovirus B19 antigens are essential for the accurate detection of parvovirus B19 IgG. J Med Virol 57:179–185

Kurtzman GJ, Cohen B, Field AM, Oseas R, Blaese RM, Young NS. 1989. Immune response to B19 parvovirus and an antibody defect in persistent viral infection. J Clin Invest 84: 1114–1123.

Manaresi E, Gallinella G, Zerbini M, Venturoli S, Gentilomi G, Musiani M. 1999. IgG immune response to B19 parvovirus VP1 and VP2 linear epitopes by immunoblot assay. J Med Virol 57:174–178.

Morinet F, D'Auriol L, Tratschin JD, Galibert F. 1989. Expression of the human parvovirus B19 protein fused to protein A in *Escherichia coli*. Recognition by IgM and IgG antibodies in human sera. J Gen Virol 70:3091–3097.

Musiani M, Azzi A, Zerbini M., Gibellini D, Venturoli S, Zakrzewska K, Re MC, Gentilomi G, Gallinella G, La Placa M. 1993. Nested polymerase chain reaction assay for the detection of B19 parvovirus DNA in human immunodeficiency virus patients. J Med Virol 40:157–160.

Musiani M, Zerbini M, Gentilomi G, Plazzi M, Gallinella G, Venturoli S. 1995. Parvovirus B19 clearance from peripheral blood after acute infection. J Infect Dis 172:1360–1363.

Ozawa K, Ayub J, Hao YS, Kurtzman G, Shimada T, Young N. 1987. Novel transcription map for the B19 (human) pathogenic parvovirus. J Virol 61:2395–2406

Ozawa K, Young NS. 1987. Characterization of capsid and noncapsid proteins of B19 parvovirus propagated in human erythroid bone marrow cell cultures. J Virol 61:2627–2630.

Palmer P, Pallier C, Leruez-Ville M, Deplanche M, Morinet F. 1996. Antibody response to human parvovirus B19 in patients with primary infection by immunoblot assay with recombinant proteins. Clin Diagn Lab Immunol 3:236–238.

Rosenfeld SJ, Yoshimoto K, Kajigaya S, Anderson S, Young NS, Field A, Warrener P, Bansal G, Collet MS. 1992. Unique region of the minor capsid protein of human parvovirus B19 is exposed on the virion surface. J Clin Inv 89:2023–2029.

Saikawa T, Anderson S, Momoeda M, Kajigaya S, Young NS. 1993. Neutralizing linear epitopes of B19 parvovirus cluster in the VP1 unique and VP1-VP2 junction regions. J Virol 67:3004–3009.

Schwartz TF, Roggendorf M, Deinhardt F. 1988. Human parvovirus B19, ELISA and immunoblot assay. J Virol Meth 20:155–168.

Söderlund M, Brown CS, Spaan WJM, Hedman L, Hedman K. 1995. Epitope type-specific IgG responses to capsid proteins VP1 and VP2 of human parvovirus B19. J Infect Dis 172:1431–1436

Söderlund M, Brown KE, Meurman O, Hedman K. 1992. Prokariotic expression of a VP1 polypeptide antigen for diagnosis by a human parvovirus B19 antibody enzyme immunoassay. J Clin Microbiol 30:305–311.

Zerbini M, Musiani M, Venturoli S, Gallinella G, Gibellini D, Gentilomi G, La Placa M. 1990. Rapid screening for B19 parvovirus DNA in clinical specimens with a digoxigenin-labeled DNA hybridization probe. J Clin Microbiol 28:2496–2499.

Zerbini M, Musiani M. 1999. Human parvoviruses. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of Clinical Microbiology, 7th ed. Washington, DC:American Society for Microbiology p 1089–1098.